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Original Article

EFFECT OF DIFFERENT DOSES OF GAMMA-IRRADIATED SODIUM ALGINATE AND NITROGEN FERTILIZER ON GROWTH, PHYSIOLOGICAL, BIOCHEMICAL PARAMETERS AND YIELD ATTRIBUTES OF *MENTHA PIPERITA* L. IN NORTHERN HIMALAYAS

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ABSTRACT

A field experiment was conducted for one year, in order to observe the combined effect of the different concentration ranges of irradiated sodium alginate (ISA), along with the different concentration doses of Nitrogen fertilizers on the overall growth and yield attributes of Mentha piperita L. for 120 days after plantation (120 DAP) in Srinagar (J&K), India. A unique concentrations of irradiated sodium alginate ISA $_{80}$ (80 mgL^{-1}) was prepared along with the combination doses of Nitrogen (N_{80}) in the form of 20 kg ha^{-1}, 40 kg ha^{-1}, 60kg ha^{-1}, 80 kg ha^{-1} and 100 kg ha^{-1} and 120 kg ha^{-1}. The total of seven treatments was prepared viz, T1 (ISA $_{80}$ + N_{20} kg ha^{-1}), T2 (ISA $_{80}$ + N_{40} kg ha^{-1}), T3 (ISA $_{80}$ + N_{60} kg ha^{-1}), T4 (ISA $_{80}$ + N_{80} kg ha^{-1}), T5 (ISA $_{80}$ + N_{100} kg ha^{-1}), T6 (ISA $_{80}$ + N_{120} kg ha^{-1}) and T7 (Control as Double Distil Water). Among various foliar spray of irradiated sodium alginate, ISA $_{80}$ (80 mg L^{-1}) along with nitrogen, N_{80} (80 kg N ha^{-1}) proved to be the best over the control for overall parameters studied including growth, physiological, biochemical and yield attributes in Mentha piperita L. Among the growth attributes, the plant height showed increase by (40.58%), fresh weight by (54.08%), dry weight by (57.89%) were as the leaf yield per plant showed the maximum increase of 146.07 % over the control in Mentha piperita L. Besides, the same trend was noticed while analyzing the physiological and biochemical parameters which showed the maximum increase of 35.71 % by application of T4 (ISA $_{80}$ + N_{80} kg ha^{-1}) in Carotenoid content over the control in Mentha piperita L. However, at T4 (ISA $_{80}$ + N_{80} kg ha^{-1}), the yield attributes like essential oil content showed the increment by (26.31%), essential oil yield by (90.00%), herbage yield by (67.38%) over the control in M. piperita L.

KEYWORDS: Irradiated Sodium Alginate & Mentha piperita L.

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INTRODUCTION

Aromatic plants, since the time immortal have been proven beneficial in terms of essential oil derivatives and other plant extracts. Polysaccharides like carrageen an, sodium alginate, chitosan are obtained from naturally occurring brown algae *Sargassum* in huge quantities. Sodium alginate, along with chitosan is known to be the elicitors for the defense mechanisms in the plants (John, Rohrig, Schmidt, Walden, & Schell, 1997; Mercier et al., 2001). Gamma irradiated Sodium Alginate has shown the tremendous growth promoting activities and behaves as a bio-fertilizer (Mollah et al., 2009). Enhanced shoot elongation, seed germination, as well as root modifications were observed by the foliar spray of gamma irradiated sodium alginate (Natsume et al., 1994; Hu *et al.*, 2004). *Mentha piperita* L. Belongs

to the family *Lamiacea* and is widely cultivated commercial crop in the northern semi-arid and sub-tropical regions of India. India is currently producing nearly 18000 tones of mint oil per year and has emerged as one of the largest supplier of mint oil and menthol (Patra et al., 2008). Menthol mint is a potential source of natural menthol and other constituents like mint terpenes, menthone, isomenthone, menthofuran which are widely used in drug industry, pharmaceutical and variety of cosmetic products. Alginates have been used in varied ways for food, pharmaceutical and drinking purposes. The present work revealed that the foliar application of ISA_{80} (80 kg ha^{-1}) significantly improve growth, physiological and biochemical attributes and menthol production in *Mentha piperita* L.

MATERIAL AND METHODS

A field experiment was conducted in the botanical garden of Kashmir university, Srinagar during the year2017. The plant materials were collected from IIIM Srinagar (J&K). Small Plantlets of *Mentha piperita* L. Were grown in the fields as well as earthen pots of botanical garden (University of Kashmir).

Table 1: Soil Profile (Srinagar J&K)

Parameters Values
Texture Sandy Loam with low amount of silt
pH 5.8 – 6.13
Conductivity dsm^{-1} 0.59
Available Nitrogen (mg/Kg Soil) 98.47
Available Phosphorous (mg/Kg Soil) 145.01
Available Potassium (mg/Kg Soil) 6.8
Soil Temperature (ºC) 12.31

Plant Materials, Location of Cultivation and Growth Conditions

The Pot experiments were conducted under controlled conditions in Botanical Garden University of Kashmir. Each earthen pot (25 cm diameter x 25 cm height) was filled with 5 Kg homogenous mixture of soil and organic manure in the ratio of 4:1. The soil analysis was done at agricultural farms soil testing unit at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K).

Irradiation and Gel Permeation Chromatography (GPC) Analysis

Solid form of sodium alginate (Sigma Aldrich, USA) was properly sealed in a long glass tube with atmospheric air. The samples of sodium alginate were irradiated in a Gamma Chamber (Cobalt-60, GC-5000) manufactured by Bhabha Atomic Research Centre (BARC, Zakura, Srinagar), India. The samples were irradiated to 520 kGy gamma radiation dose at a dose rate of 2.4 kGy/h. various aqueous concentrations of irradiated sodium alginate (ISA) were prepared.

Estimation of Growth Parameters

Plant materials were brought from IIIM (Srinagar). The field trials were conducted during 2016-2017. The experiments were conducted in randominised block design in earthen pots (25 cm height x 25cm diameter). The plants were sown in the month of April. At least three replicates of each treatment were planted in the fields. The doses of irradiated sodium alginate (ISA) and Nitrogen (N) were selected on the basis of earlier studies Aftab et al., (2011 a, b). The plants were irrigated as and when required. The treatments consisted of Control (foliar spray of double distil water) along with the Irradiated Sodium Alginate concentration i.e. 80 mgL^{-1} (ISA_{80}), and Nitrogen doses in the form of 20 kg ha^{-1} 40 kg ha^{-1},60kg ha^{-1},80 kg ha^{-1} and 100 kg ha^{-1} and 120 kg ha^{-1}. The first foliar spray was given to the plants after 40 days of

plantation (DAP). Five foliar sprays were given to the plants under experimentation. (Table 2)

Estimation of Physiological and Biochemical Parameters

Determination of Chlorophyll Content and Carotenoid Content

Total content of leaf chlorophyll and carotenoids was estimated using the method of Lichtenthaler and Buschmann (2001) The fresh tissue from the interveinal area of leaf was grinded with 100% acetone using mortar-pestle. The optical density (OD) of the pigment solution was recorded at 662, 645 and 470 nm to determine chlorophyll a, chlorophyll b and total carotenoids content, respectively, using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). Total chlorophyll content was assessed by adding the contents of chlorophyll a and b. The content of photosynthetic pigments was expressed as mg g^{-1} leaf FW. (Table 3)

Determination of Carbonic Anhydrase (CA) Activity

The activity of carbonic anhydrase was measured in the fresh leaves, using the method described by Dwivedi and Randhawa (1974). Two hundred mg of fresh leaves (chopped leaf pieces) was transferred to Petri plates. The leaf pieces were dipped in 10 mL of 0.2 M Cystein hydrochloride solution for 20 min at 4 ◦C. The solution adhering at the cut surfaces of the leaf pieces was then removed with the help of a blotting paper followed by their transfer immediately to a test tube containing 4 mL of phosphate buffer ofpH6.8. To it, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.022% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme activity was expressed as M CO2 kg^{-1} leaf FWs^{-1}. (Table 3)

Total Phenol Content

Total phenol content was estimated by the method described by Sadasivam and Manickam (2008). Five hundred mg of the leaves were grinded with 10 times volume of 80% ethanol, using mortar pestle. The homogenate was centrifuged at 10,000 rpm (10,062 × g) for 10 min at 4 °C. The supernatant was evaporated to dryness, adding 5 mL of double distilled water (DDW) thereafter. Later, 0.5 mL of Folin-Ciocalteau Reagent and 2 mL of 20% Na_2CO_3 solution were added to each test tube. The OD of the solution, thus obtained, was measured at 650 nm against a reagent blank. Using the standard curve, the content of total phenols in the test samples was determined as mg phenol per 100 g of dry leaves. (Table 3)

Estimation of N, P and K Contents in Leaves

Leaf samples from each treatment were digested for the estimation of leaf-N, -P and -K contents. The leaves were dried in a hot air oven at 80 ◦C for 24 h. The dried leaves were ground using mortar and pestle and the leaf-powder was passed through a 72 mesh. The sieved leaf-powder was used for the estimation of N, P and K contents. One hundred mg of the oven-dried leaf powder was carefully transferred into a digestion tube, to which 2 mL of AR (analytical reagent) grade concentrated sulfuric acid was added subsequently. This solution was heated on a temperature controlled assembly at 100 ◦C for about 2 h and then the content was cooled for about 15 min at room temperature. To the cooled content, 0.5 mL of 30% hydrogen peroxide () was added drop by drop. The addition of H_2O_2 was followed by gentle heating of the content; then, the content was cooled at room temperature. This step was repeated until the content of the tube turned colorless. The aliquot (peroxide-digested material), thus prepared, was used to estimate the per cent N, P and K contents in the leaves on dry weight basis. (Table 3)

Determination of N Content

Leaf-N content was estimated according to method of Lindner (1944) with slight modification by Novozamsky, Houba, van Eck, and van Vark (1983). The dried leaf-powder samples were digested with H2SO4 in the digestion tubes using temperature controlled Kjeldahl assembly. A 10 mL aliquot (peroxide-digested material) was poured into a 50 mL volumetric flask. To it, 2 mL of 2.5 N Sodium Hydroxide and 1 mL of 10% sodium silicate solutions were added to neutralize the excess acid and prevent turbidity. A 5 mL aliquot of the peroxide-digested plant material was poured into a 10 mL graduated test tube followed by addition of 0.5 mL of Nessler's reagent. The OD (optical density) of the solution was recorded at 525 nm using the spectrophotometer. (Table 3)

Determination of P content

The method of Fiske and Subba Row (1925), with slight modification by Rorison, Spencer, and Gupta (1993) was used to estimate the leaf-P content in the peroxide-digested material. A 5 mL aliquot was poured into a 10 mL graduated test tube. To it, 1 mL of molybdic acid (2.5%) was added, followed by addition of 0.4 mL of 1-amino-2-naphthol-4-sulfonic acid. When the color of the content turned blue, the volume of the test tube was made up to 10 mL, using double distilled water. The OD of the solution was recorded at 620 nm using the spectrophotometer. (Table 3)

Determination of K Content

Leaf-K content was determined in the peroxide digested plant material by a flame-photometer (Hald, 1947) (Model, C150, AIMIL, India) with the help of emission spectra using specific filter. In the flame-photometer, the solution (peroxide-digested material) was discharged through an atomizer in the form of a fine mist into a chamber, where it was drawn into a flame. Combustion of the elements produced the light of a particular wavelength [max for K = 767 nm (violet)]. The light, thus produced, was passed through an appropriate filter to impinge upon a photoelectric cell that subsequently activated a galvanometer in order to get the reading. (Table 3)

Estimation of Yield Attributes

Estimation of Essential Oil Content and Yield

After attaining full maturity at 120 days of plantation the crops were subjected to harvesting process. From each treatment 500g of fresh plant parts including leaves were taken and crushed. Oil extraction process was estimated according to the (Guenther 1972). Considerable amount of chopped leaves were taken into account for EO estimation and EO content was extracted through Clevenger's apparatus used for three hours. (Table 4).

Statistical Analysis

The data were analyzed statistically using SPSS- 17 statistical software (SPSS Inc., Chicago, IL, USA). Means were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. Standard errors were taken into consideration.

RESULTS

The present study revealed that the spray of Irradiated Sodium Alginate and Nitrogen fertilizers applied to the plants improved the performance of *Mentha piperita* L. crop significantly. Further, this study revealed that the combined application of Irradiated Sodium Alginate and Nitrogen fertilizers gave the better performance on the overall growth attributes, physiological and biochemical attributes as well as essential oil composition and essential oil yield in *Mentha*

piperita L. as compared to the control. (Table 2,3 and 4). Out of the seven spray treatments, the combination of doses ISA_{80} + N_{80} (T4) proved to be the optimal as compared to the control. The details are as follows:

Growth Attributes

As compared to the control, the significant effect of foliar spray of different treatments was proved to be beneficial for overall growth of the plant i.e. plant height, fresh weight, dry weight of the plants, root length shoot length, leaf area per plant as well as leaf yield per plant. Among the various combined treatments of the plant growth regulators T4 i.e. (ISA_{80} (80 mg L^{-1}) + N_{80} (80 kg N ha^{-1}), the plant height was increased by 40.58%, fresh weight and dry weights of the plants showed increase by 54.08% and 57.89%, shoot length and root length per plant by 45.33% and 131.50 % respectively and leaf- area and leaf - yield per plant by 68.63 % and 146.07 % respectively over the control in *Mentha piperita* L. Like other plant growth regulators, Alginate Oligosaccharides (AO's) resemble the growth elicitors that initiate the biosynthesis of various enzymes including the activation of gene expressions (Ma. Li,Bu & Li, 2010). The influence of natural polysaccharides, such as carrageen an, sodium alginate and chitosan has proved to be significant for plant growth parameters (Abad et al., 2009; Aftab et al., 2011; Hegazy, Abdel Rehim, Diaa, & El-Barbary, 2009; Hien et al., 2000; Hu, Jiang, Hwang, Liu, & Guan, 2004; Khan, Khan, Aftab, Idrees, & Naeem, 2011; Kume et al., 2002; Luan et al., 2003; Mollah, Khan, & Khan, 2009; Natsume, Kamao, Hirayan, & Adachi, 1994; Relleve et al., 2000; Sarfaraz et al., 2011; Tomoda, Umemura, & Adachi, 1994).

Physiological and Biochemical Attributes

Photosynthetic Pigments

As far as chlorophyll content is concerned, the application of depolymerized form of ISA at T4 (ISA_{80} (80 mg L^{-1}) along with nitrogen, N_{80} (80 kg N ha^{-1}) significantly increased the total content of chlorophyll by (31.48%) and that of carotenoids (35.71%) at 120 DAP as compared to the T7 control (Table 3).

Nitrate Reductase (NR) Activity

ISA applied along with Nitrogen doses, increased the NR activity maximally. The increase in NR activity by (23.15 %) was shown by the T4 (ISA_{80} (80 mg L^{-1}) along with nitrogen, N_{80} (80 kg N ha^{-1}) over the control T7 in *Mentha piperita* L. (Table 3).

Carbonic Anhydrase Activity

The application of T4 (ISA_{80} (80 mg L^{-1}) along with nitrogen, N_{80} (80 kg N ha^{-1}) proved optimum for the CA activity at 120 DAP. It registered 26.79% higher activity of the enzyme compared to the control T7 (Table 3).

Leaf - N -P and -K Contents

Leaf -N, -P and -K contents were also significantly enhanced by combined application ISA and Nitrogen doses with T4 (ISA_{80} (80 mg L^{-1}) along with nitrogen, N_{80} (80 kg N ha^{-1}) proving the best. It increased the leaf -N, -P and -K content by 47.42%, 4.16%, and 11.59%, respectively, over the control at 120 DAP (Table 3).

Total Phenolic Content

Total phenol content was significantly increased by the combined effect of ISA and Nitrogen doses with T4 (ISA_{80} (80 mg L^{-1}) along with nitrogen, N_{80} (80 kg N ha^{-1}) proving to be the optimal with 31.42 % increase over the control T7

in *Mentha piperita* L. (Table 3).

Yield Attributes

Essential oil content was significantly increased by the combined effect of ISA and Nitrogen doses. The best response was seen in T4(ISA $_{80}$ (80 mg L^{-1}) along with nitrogen, N_{80} (80 kg N ha^{-1}) which showed the enhancement of EO by (26.31%), essential oil yield by (90.00%), herbage yield by (67.38%) respectively as compared to the control in *Mentha piperita* L. (Table 4).

Table 2: Effect of Gamma – Irradiated Sodium Alginate and Nitrogen Fertilizers on the Growth Attributes *of Mentha Piperita L* after 120 Days of Plantation

Growth Attributes	Treatment Concentrations						
	T1 $ISA_{80}+N_{20}$ ISA_{80} (80 Mg L^{-1}) + N_{20} (20 Kg N Ha^{-1})	T2 $ISA_{80}+N_{40}$ ISA_{80} (80 Mg L^{-1}) + N_{40} (40 Kg N Ha^{-1})	T3 $ISA_{80}+N_{60}$ ISA_{80} (80 Mg L^{-1}) + N_{60} (60 Kg N Ha^{-1})	T4 $ISA_{80}+N_{80}$ ISA_{80} (80 Mg L^{-1}) + N_{80} (80 Kg N Ha^{-1})	T5 $ISA_{80}+N_{100}$ ISA_{80} (80 Mg L^{-1}) + N_{100} (100 Kg N Ha^{-1})	T6 $ISA_{80}+N_{120}$ ISA_{80} (80 Mg L^{-1}) + N_{120} (120 Kg N Ha^{-1})	T7 Control (DDW)
Plant Height (cm)	94.40 ± 1.68^{g}	99.10 ± 2.89^{d}	107.21 ± 1.18^{c}	124.41 ± 0.85^{a}	110.72 ± 2.58^{bc}	121.20 ± 1.85^{b}	88.21 ± 1.41^{f}
Shoot Length (cm)	20.60 ± 1.26^{d}	21.87 ± 2.14^{d}	22.00 ± 1.20^{c}	27.12 ± 3.42^{a}	24.43 ± 1.21^{b}	24.10 ± 2.28^{b}	18.66 ± 1.21^{g}
Root Length (cm)	9.41 ± 1.44^{f}	10.66 ± 1.81^{g}	11.69 ± 1.71^{d}	15.21 ± 1.28^{a}	14.01 ± 1.34^{b}	12.78 ± 1.21^{c}	6.57 ± 1.71^{g}
Leaf-area per plant (cm^2)	1824.39 ± 3.34^{f}	1919.31 ± 19.85^{g}	2183.15 ± 33.55^{d}	3004.85 ± 21.92^{a}	2447.04 ± 2.48^{c}	2456.06 ± 13.76^{b}	1781.91 ± 3.80^{g}
Leaf-yield per plant (g)	8.45 ± 0.42^{f}	9.01 ± 0.26^{g}	9.65 ± 0.36^{d}	12.07 ± 0.96^{a}	10.04 ± 0.81^{b}	9.88 ± 0.65^{c}	4.08 ± 0.49^{g}
Fresh Weight per plant (g)	37.41 ± 2.33^{de}	39.50 ± 1.32^{cd}	43.70 ± 1.41^{bc}	52.85 ± 1.27^{a}	44.44 ± 1.04^{b}	47.17 ± 1.63^{b}	34.30 ± 1.21^{g}
Dry Weight per plant(g)	10.21 ± 0.15^{f}	10.70 ± 0.20^{g}	11.42 ± 0.15^{d}	15.10 ± 0.58^{a}	12.21 ± 0.17^{c}	13.01 ± 0.12^{b}	9.50 ± 0.17^{g}

Table 3: Effect of Gamma – Irradiated Sodium Alginate and Nitrogen Fertilizers on the physiological and Biochemical Attributes *of Mentha Piperita* L. after 120 Days of Plantation

Physiological And Biochemical Attributes	Treatment Concentrations						
	T1 $ISA_{80}+N_{20}$ ISA_{80} (80 Mg L^{-1}) + N_{20} (20 Kg N Ha^{-1})	T2 $ISA_{80}+N_{40}$ ISA_{80} (80 Mg L^{-1}) + N_{40} (40 Kg N Ha^{-1})	T3 $ISA_{80}+N_{60}$ ISA_{80} (80 Mg L^{-1}) + N_{60} (60 Kg N Ha^{-1})	T4 $ISA_{80}+N_{80}$ ISA_{80} (80 Mg L^{-1}) + N_{80} (80 Kg N Ha^{-1})	T5 $ISA_{80}+N_{100}$ ISA_{80} (80 Mg L^{-1}) + N_{100} (100 Kg N Ha^{-1})	T6 $ISA_{80}+N_{120}$ ISA_{80} (80 Mg L^{-1}) + N_{120} (120 Kg N Ha^{-1})	T7 Control (DDW)
Chlorophyll Content	94.40 ± 1.68^{g}	99.10 ± 2.89^{d}	107.21 ± 1.18^{c}	124.41 ± 0.85^{a}	110.72 ± 2.58^{bc}	121.20 ± 1.85^{b}	88.21 ± 1.41^{f}
Carotenoid Content	0.28 ± 0.01^{f}	0.29 ± 0.01^{g}	0.33 ± 0.02^{d}	0.42 ± 0.02^{a}	0.39 ± 0.02^{b}	0.36 ± 0.02^{c}	0.26 ± 0.01^{g}
Nitrate Reeducates activity	217.05 ± 2.08^{f}	229.71 ± 2.46^{g}	264.11 ± 2.04^{b}	275.01 ± 1.56^{a}	251.07 ± 1.50^{c}	245.62 ± 1.74^{d}	210.62 ± 1.07^{g}
Carbonic Anhydrase activity	210.07 ± 1.82^{f}	219.37 ± 1.62^{g}	226.72 ± 2.77^{d}	255.71 ± 1.49^{a}	247.30 ± 2.82^{b}	231.59 ± 2.49^{c}	203.63 ± 2.29^{g}
Total phenolic content (mg g^{-1})	1.49 ± 0.01	1.50 ± 0.02	1.70 ± 0.01	1.84 ± 0.02	1.56 ± 0.01	1.75 ± 0.01	1.40 ± 0.02
Leaf Nitrogen Content (%)	3.10 ± 0.71^{f}	3.18 ± 0.69^{g}	3.26 ± 0.81^{d}	4.29 ± 1.32^{a}	4.04 ± 1.20^{b}	3.73 ± 1.41^{c}	2.91 ± 1.00^{g}
Leaf Phosphorous Content (%)	0.24 ± 0.002^{b}	0.24 ± 0.002^{b}	0.24 ± 0.002^{b}	0.25 ± 0.003^{a}	0.25 ± 0.003^{a}	0.25 ± 0.003^{a}	0.24 ± 0.002^{b}
Leaf Potassium Content (%)	2.13 ± 0.24^{f}	2.16 ± 0.21^{g}	2.22 ± 0.24^{d}	2.31 ± 0.42^{b}	2.28 ± 0.79^{a}	2.23 ± 0.31^{c}	2.07 ± 0.57

Table 4: Effect of Gamma – Irradiated Sodium Alginate and Nitrogen Fertilizers on the Yield Attributes of *Mentha Piperita* L after 120 Days of Plantation

Yield Attributes	Treatment Concentrations						
	T1 $ISA_{80}+N_{20}$	T2 $ISA_{80}+N_{40}$	T3 $ISA_{80}+N_{60}$	T4 $ISA_{80}+N_{80}$	T5 $ISA_{80}+N_{100}$	T6 $ISA_{80}+N_{120}$	T7 (DDW) Control
Herbage- Yield per Plant (cm)	30.11 ± 1.29^{c}	37.21 ± 1.30^{b}	39.31 ± 0.97^{b}	45.70 ± 2.58^{a}	41.51 ± 3.13^{b}	40.21 ± 1.39^{b}	27.31 ± 0.94^{d}
Essential- Oil Content (%)	0.39 ± 0.01^{b}	0.40 ± 0.01^{b}	0.39 ± 0.01^{b}	0.48 ± 0.01^{a}	0.31 ± 0.01^{c}	0.33 ± 0.02^{c}	0.38 ± 0.01^{b}
Essential-Oil Yield (ml)	0.11 ± 0.02^{cd}	0.14 ± 0.01^{bc}	0.15 ± 0.01^{b}	0.19 ± 0.01^{a}	$0.13 \pm 0.^{02bcd}$	0.12 ± 0.01^{bcd}	0.10 ± 0.01^{d}

DISCUSSIONS

Growth Attributes

The data presented in (Table 2) indicated that combined effect of ISA and Nitrogen doses have promotive effects on all the growth parameters studied. The foliar application of T4 (ISA_{80} + N_{80}) exhibited the significant response to enhance the growth attributes as compared to the control plants T7 (Double Distil Water). These results are in significance with those of Hein et al., 2000) who reported that gamma – irradiated SA, applied at concentration of 20-100 ppm, enhanced the productivity of carrot, cabbage, barley, rice, peanut, tea during the field investigations. Alginate derived oligosaccharides promoted the biomass or dry weight of tomato seedling (Ruizhi et al., 2009). According to El-Rehim (2006) and Mollah et al. (2009), the oligomers derived from de-polymerization of alginate released from the copolymer were absorbed by plants. The absorption of oligomers acted as a growth promoter, which resulted in elongation of plant root and shoot of plants and, in turn, resulted in an increase in plant productivity and significant improvement in physiological parameters compared with the untreated plants. They maintained that ISA might have growth promoting activities like other plant growth promoters and could act as a bio-fertilizer. Exogenous factors include various plant growth promoters, which have direct or indirect influence on growth and development of the plants. The effect of irradiated natural polysaccharides, such as sodium alginate, carrageenan and chitosan has been reported positive for plant growth parameters in various earlier studies (Natsume, Kamao, Hirayan, & Adachi,1994; Tomoda, Umemura, & Adachi, 1994; Hien et al., 2000; Relleve et al., 2000; Kume et al., 2002; Luan et al., 2003; Hu, Jiang, Hwang, Liu, & Guan, 2004; Abad et al., 2009; Mollah et al., 2009; Jamsheer, 2010; Qureshi, 2010; Sarfaraz et al., 2011; Khan et al., 2011; Aftab et al., 2011, 2013; Naeem et al., 2011, 2012; Idrees et al., 2012).

Physiological and Biochemical Attributes

Photosynthetic Pigments

Increment in the chlorophyll and carotenoid content due to the combined application of ISA and Nitrogen doses (Table 3) might be ascribed to a favorable effect of ISA and Nitrogen doses on photosynthesis, as well as on the overall growth of the plant. In fact, various workers have reported positive effect of ISA and N on the total content of chlorophyll and carotenoids in the leaves of artemisia (Aftab et al., 2013), lemongrass (Idrees et al., 2012), mentha (Naeem et al., 2011, 2012), fennel (Sarfaraz et al., 2011), red amaranth (Mollah et al., 2009) opium poppy (Khan et al., 2011). The ISA has also been reported to induce cell signaling, leading to stimulation of various physiological processes in various plants, including ISA-mediated improvement in the content of photosynthetic pigments and net photosynthetic rate (Farmer, Thomas, Michael, & Clarence, 1991).

NR Activity

A significant increase in leaf-NR activity by ISA and N in this study might be related to the increase in leaf-N and -P content that might have possibly increased the nitrate concentration in leaves. In fact, the substrate (nitrate) concentration essentially induces functional NR activity (Hewitt & Afridi, 1959) by producing nitrate sensing protein of unknown nature (Campbell, 2002). The results of the present study are concordant with those obtained regarding artemisia (Aftab et al., 2013), lemongrass (Idrees et al., 2012), and in the case of fennel (Sarfaraz et al., 2011), and those of regarding opium poppy (Khan et al., 2011). The positive effect of ISA and N concentration on NR activity has also been reported by Naeem et al., 2011; in the case of *Mentha arvensis* L.

Determination of Carbonic Anhydrase (CA) Activity

CA is one of the most abundant zinc containing proteins in plants. It has an active role in photosynthesis, which is evident by its presence in all photosynthesizing tissues. It catalyzes the reversible hydration of CO2 to carbonic acid, thereby increasing the availability of CO2 to RuBisCO in photosynthesis (Badger & Price, 1994). The application of ISA $_{80}$ + N_{80} mg L^{-1} proved optimum for the CA activity. It registered 26.79% % higher activity of the enzyme compared to the control (Table 3). Such a plant response to ISA and doses of Nitrogen is expected because, the deploymerized natural Polysaccharides have been reported to increase the stomatal conductance significantly (Naeem et al., 2011), which might facilitate the diffusion of additional amounts of CO_2 through the stomata to be acted upon by CA, resulting in the enhanced CA activity. Further, a probable reason for the enhancement of CA activity could be the ISA-mediated de novo synthesis of CA, which might involve transcription/translation of the genes associated as has been reported for other degraded natural polysaccharides (Knowles & Ries, 1981). Expectedly, the enhancement of CA activity in the combined application of ISA and N treated plants might be responsible for the enhanced rate of CO_2 fixation (not measured in this study) that could have resulted in significant increase in fresh and dry weights of the plants (Table 2). Regarding ISA -mediated CA activity, our findings are similar to those that claim the synthesis of certain enzymes in the tissue culture, as a result of application of irradiated natural polysaccharides (Akimoto, Aoyagi, & Tanaka, 1999; Patier et al., 1995).

Total Phenolic Content

The ISA alone increased the level of leaf phenolic content at each sampling stage. However, combined effect of ISA with N was much pronounced increasing the level of phenolic content in the leaves at 120 DAP (Figure 3). The positive results obtained in this regard, in response to ISA application might be ascribed to such a specific role of oligomers obtained by irradiation with Co-60 gamma rays. In addition, Nitrogen dose combination doses of ISA along with N improved the leaf-phenolic content at the sampling stages. The leaf phenolic content reflects the free radical scavenging capability of the plant that may help the plant to maintain the normal growth at later growth stages, at which frequent production of free radicals takes place, inducing the bad effects of aging (Dimitrios, 2006). The significant effect of ISA on phenol content has also been reported by Naeem et al. (2011) regarding mint respectively.

CONCLUSIONS

The application of irradiated sodium alginate resulted in significant improvement in growth attributes, physiological and biochemical parameters in addition to enhancing the essential oil content and essential oil yield along with the herbage yield. The best ISA concentration proved to be the ISA $_{80}$ + N_{80}. These findings corroborate the earlier findings carried out regarding the effect of other irradiated natural polysaccharides on growth, yield and quality of other medicinal and aromatic crops. However, ISA dependent enhancement of growth, yield and quality of *Mentha piperita* L. has been worked out for the first time in this investigation. Further, this research may also instigate the scientists to find out the optimum concentration of ISA or other irradiated natural polysaccharides for different medicinal and aromatic plants in order to enhance the productivity, quality and production of EO and other active constituents. Furthermore, the technique is highly safe as we used the gamma radiations only to depolymerize the sodium alginate rather exposing the plants or seeds. In addition to that, this is one step processing method to obtain the oligosaccharides. Above that, the application of ISA and Nitrogen doses used in this study is very economical and beneficial.

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